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Possible Influence of the Mutant CCR5 Allele on Vertical Transmission of HIV-1

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A possible correlation between the rate of vertical transmission of HIV-1 and the presence of the defective HIV co-receptor gene Δ32ccr5 in the chromosomes of infants born to HIV-positive mothers was assessed. The prevalence and genotypic distribution of the Δ32ccr5 gene were studied in 451 uninfected and 225 HIV-1-infected adults and 79 children born to HIV-1-positive mothers in Austria (45 uninfected and 34 infected by vertical transmission). As expected in a Caucasian population, the Δ32ccr5 allele was found in uninfected Austrians at a frequency of 10% (17.3% heterozygotes and 1.3% Δ32ccr5/Δ32ccr5 homozygotes, consistent with the expected Hardy-Weinberg distribution). The mutant allele frequency was 11.1% in uninfected children (17.8% heterozygotes, 2.2% homozygotes) and 9.6% in HIV-positive adults (19.1% heterozygotes but no Δ32ccr5/Δ32ccr5 homozygotes). Among the group of 34 vertically infected children, however, there were only two heterozygotes and no Δ32ccr5/Δ32ccr5 homozygotes, corresponding to a significantly reduced mutant allele frequency of 2.9% ($P = 0.05$ compared to HIV-negative children). These results suggest that CCR5/Δ32ccr5 heterozygous children are less susceptible to vertical transmission of HIV-1. The data also support the hypothesis that Δ32ccr5 homozygous individuals are resistant to HIV-1 infection. ***J. Med. Virol. 55:51–55, 1998.***

KEY WORDS: co-receptor CCR5; vertical transmission; resistance to HIV-1

INTRODUCTION

The recent discovery that in addition to the CD4 molecule so-called co-receptor molecules are involved in the cellular uptake of HIV has added a new dimension to the understanding of HIV transmission, tropism, and pathogenesis [Doranz et al., 1997; He et al., 1997]. The chemokine receptor CCR5 was identified as the major co-receptor used by macrophage-tropic (M-tropic) HIV-1 [Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996]. M-tropic strains are probably responsible for most initial HIV-1 transmissions and are predominant during early infection. Although other chemokine receptors can also function as co-receptor molecules (CCR3 and CCR2b for M-tropic, and CXCR-4 [fusin] for dual-tropic and T-tropic strains of HIV) [Doranz et al., 1996; Feng et al., 1996; Simmons et al., 1996], there is increasing and convincing evidence that CCR5 plays a crucial role in establishing and/or maintaining a permanent HIV infection [Dean et al., 1996; Huang et al., 1996; Liu et al., 1996, Samson et al., 1996].

In recent studies, a mutant allele of the CCR5 gene bearing a 32-bp frameshift deletion mutation (designated Δ32ccr5) has been found at an allelic frequency of approximately 10% in several Caucasian populations in Europe and North America, but it has not been reported to be present in any non-Caucasian populations sampled to date. Thus, among Caucasians, approximately 18% of individuals are heterozygous and 1% are homozygous for this mutation [Liu et al., 1996; Samson et al., 1996; Zimmerman et al., 1997]. Δ32ccr5 homozygotes do not express CCR5 on the cell surface but show no recognised clinical impairment due to this defect [Liu et al., 1996; Zimmerman et al., 1997]. Increasing experimental and epidemiological data suggest that these individuals may be resistant to sexually and parenterally transmitted HIV-1 [Dean et al., 1996; Huang et al., 1996; Liu et al., 1996; Samson et al., 1996; Zimmerman et al., 1997; Rana et al., 1997].

Given the high proportion of individuals heterozygous for the Δ32ccr5 mutation, it would be interesting to know whether this genotype may also confer some form of protection against HIV infection. It has been speculated that heterozygotes may express lower CCR5 protein levels and that this may affect the susceptibility to primary infection as well as reduce the rate of

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viral cell-to-cell spread in the body. Several studies indeed have provided evidence for a slower disease progression in infected heterozygous individuals [Dean et al., 1996; Huang et al., 1996; Zimmerman et al., 1997; Eugen-Olsen et al., 1997]. A partial protective effect of this genotype against primary infection was suggested by one study [Samson et al., 1996] but not confirmed by others [Dean et al., 1996; Huang et al., 1996; Zimmerman et al., 1997]. An investigation of groups of individuals infected by a uniform transmission mode at known time points may contribute to clarifying this question.

Vertical transmission is the major route of infection in children and is increasingly important due to the rapidly growing number of HIV-infected women. Factors that may influence the risk of vertical transmission, which on the average occurs in about 20%–30% of the cases, have been studied intensively to provide a basis for intervention strategies [European Collaborative Study 1996; Landesman et al., 1996; Mayaux et al., 1997; Tovo et al., 1996; Kuhn et al., 1997]. The influence of the CCR5 genotype on the rate of vertical transmission has not yet been studied. It is reasonable to hypothesise that mothers heterozygous for the Δ32ccr5 mutation may be less infectious, e.g., due to a lower viral burden, and/or that infants who are homo- or heterozygous for the Δ32ccr5 allele may be (partially) resistant to infection.

In this study, a possible correlation between the infant CCR5 genotype and the rate of vertical HIV-1 transmission was investigated. The CCR5 genotypes of HIV-infected and uninfected children born to HIV-positive mothers were examined and the frequency of the Δ32ccr5 mutant allele in the general population and in HIV-infected adults in Austria was compared.

MATERIALS AND METHODS

Subjects

Samples from four different groups of individuals from Austria were included in this study: 451 uninfected adults; 225 HIV-infected adults, including haemophiliacs, intravenous drug users, and persons infected with HIV by homosexual or heterosexual contacts; 45 uninfected children born to HIV-positive mothers; and 34 children infected vertically. The latter group is an almost complete representation of all infected children born in Austria since the beginning of the HIV epidemic (since samples from HIV-infected children in Austria are sent to the Institute of Virology for diagnostic confirmation). In the case of the control group, the samples available for this study represent an estimated one-third of all uninfected infants born to HIV-positive mothers in Austria. Three children born to mothers of African origin were excluded from the study. Breast-feeding was not considered as a factor in this study because HIV-positive mothers in Austria are generally advised not to breast-feed their infants. Samples from mothers were available in only 12 cases, and these are included in the group of infected adults.

The infection status in adults was determined by enzyme-linked immunosorbent assay (ELISA; Abbott HIV1/HIV2 third generation plus EIA; North Chicago, IL) and confirmed by Western blot assay (Sanofi Pasteur, Marnes la Coquette, France). The diagnosis of HIV infection in children was based on persistence of HIV-specific antibodies beyond 18 months [CDC, 1994] or by a positive polymerase chain reaction (PCR) result (Amplicor HIV-1; Hoffmann-La Roche, Nutley, NJ; or performed as described previously [Stoeckl et al., 1989]) from a sample taken at 3 months of age or later [Kuhn et al., 1997; Dunn et al., 1995; Kovacs et al., 1995; Krivine et al., 1992], which was confirmed by at least one additional positive PCR result or a positive p24 antigen ELISA (Abbott) from samples obtained independently. The criteria applied to exclude vertical infection were either the complete loss of HIV-specific antibodies or negative PCR results beyond 3 months of age [Dunn et al., 1995] and the absence of a positive PCR or p24 ELISA result at any time.

Determination of CCR5 Genotypes

Genomic DNA was purified from peripheral blood mononuclear cells (PBMCs) by standard methods. A region of the CCR5 gene spanning the site of the 32-bp deletion in mutant alleles was then amplified by PCR using primers CCR5c (5′-CAAAAAGAAGGTCTTCATTACACC-3′) and CCR5d (5′-CCTGTGCCTCTTCTTCTCATTTCG-3′), as described by Huang et al. [1996]. These primers yield fragments of 189- and 152-bp length for the wild-type and mutant CCR5 genes, respectively, which were distinguished after fractionation on 3% NuSieve gels. The identity of the bands observed was confirmed by automated direct nucleotide sequence analysis of the PCR fragments (373A sequencer; Perkin-Elmer, Oak Brook, IL). To further confirm homozygous phenotyes, semi-nested PCRs using primers specific for wild-type (CCR5w, 5′-CAGCTCTCATTTTCCATACAGTC-3′) or Δ32ccr5 mutant (CCR5m, 5′-CAGCTCTCATTTTCCATACATTA-3′) sequences were performed.

Statistical Analysis

The Δ32ccr5 mutant allele frequencies were compared by 2 × 2 contingency tables yielding χ^2 with 1 degree of freedom and corresponding P values. In addition, P values were calculated using Fisher's exact test. Standard errors of allelic frequencies were calculated as $\sqrt{p(1 - p)/N}$ (N = total number of alleles, p = allelic frequency expressed as a proportion instead of as a percentage).

RESULTS

The CCR5 genotypes of 451 uninfected control individuals, 225 HIV-positive adults, and 79 children born to HIV-positive mothers were determined as summarised in Table I. The results obtained for the uninfected control group indicate that the genotypic distribution in Austria matches that reported for other Caucasian populations, which was found in several studies

TABLE I. Numbers and Frequencies of CCR5 Genotypes From Sample Population Groups in Austria

	Number of individuals (%)			
Group	Total	CCR5/CCR5	CCR5/Δ32ccr5	Δ32ccr5/Δ32ccr5
Adults				
Uninfected	451	367 (81.4)	78 (17.3)	6 (1.3)
Infected[a]	225	182 (80.9)	43 (19.1)	0
Male	159	127 (79.9)	32 (20.1)	0
Female	46	39 (84.8)	7 (15.2)	0
Children				
Uninfected	45	36 (80.0)	8 (17.8)	1 (2.2)
Infected	34	32 (94.1)	2 (5.9)	0

[a]Females include 12 mothers of 17 children included in this study. Gender of the remaining 20 HIV-infected individuals unknown.

TABLE II. Comparison of Δ32ccr5 Mutant Allele Frequencies

Group	Total number of alleles	Δ32ccr5 frequency (%)	Standard error (%)	χ^2	P[a]
Infected children	68	2.9	2.0	—	—
Uninfected children	90	11.1	3.3	3.68	0.055 (0.049)
Infected adults	450	9.6	1.4	3.26	0.071 (0.047)
Uninfected adults	902	10.0	1.0	3.65	0.056 (0.033)

[a]In parentheses, one-tailed P values determined by Fisher's exact test.

to be around 20% heterozygotes and 1% Δ32ccr5 homozygotes.

Among the 225 HIV-infected adults, none was found to be homozygous for the Δ32ccr5 allele. The proportion of heterozygotes, however, was similar to the uninfected control group (19.1% compared to 17.3%), thus providing no evidence for a partial protective effect of heterozygosity in this group of adult HIV-positive individuals. Furthermore, no significant difference in genotypic distributions between HIV-infected men and women was revealed (Table I).

The group of uninfected children born to HIV-positive mothers exhibited no major difference from the pattern of the uninfected adult control group (17.8% heterozygotes and 2.2% [one child] homozygous for the Δ32ccr5 mutant allele). In contrast, none of the children infected vertically was homozygous for the mutant allele and only two children (5.9%) contained one copy of the Δ32ccr5 allele. Thus, the percentage of heterozygotes in the group of infected children is considerably lower than in the other three groups.

In order to evaluate the significance of this difference, the Δ32ccr5 mutant allelic frequencies were calculated. As summarised in Table II, the mutant allele frequencies were found to be about 10% in both uninfected and infected adults, approximately 11% in the group of uninfected children born to HIV-positive mothers, and only 2.9% in the group of infected infants. The P values calculated for the numbers of wild-type and mutant alleles present in this latter group compared to each of the other three groups were close to 0.05, indicating statistical significance.

Due to the fact that this is a retrospective analysis, we were successful only in a limited number of cases in determining mother–child relationships and establishing the maternal genotypes. This was possible for 12 HIV-positive mothers included in the group of HIV-positive adults and their 17 children (22% of children included in the study). These data are presented on an individual level in Table III. Two of these mothers (16.7%) had heterozygous genotypes. Each of their children (one homozygous for the wild-type allele, the other homozygous for the mutant Δ32ccr5 allele) remained uninfected. All three heterozygous children included in this comparison of the mother–child pairs remained uninfected. This is particularly interesting in the case of mother 6 (Table III) because the first child of this woman was homozygous for the wild-type CCR5 allele and became infected vertically, whereas her second child (born 3 years later) was heterozygous and remained uninfected.

DISCUSSION

The issue of co-receptors of HIV infection and the implications for the pathogenesis and control of AIDS has become a major focus of research [Doranz et al., 1997; D'Souza and Harden, 1996]. The finding that a deletion in the gene for the co-receptor CCR5 exhibited by a proportion of Caucasian, but not non-Caucasian, populations may confer resistance to infection offers new approaches for prevention and therapy [Dean et al., 1996; Huang et al., 1996; Liu et al., 1996; Samson et al., 1996; D'Souza and Harden, 1996].

The prevalence and distribution of this genetic CCR5 defect were studied in Austria, a central European country of essentially uniform Caucasian genetic heritage. In good agreement with data published by others [Liu et al., 1996; Samson et al., 1996; Zimmerman et al., 1997], we determined in the uninfected Austrian population a frequency of the mutant allele of 10% and

TABLE III. Genotypes and HIV-Infection Status of Individual HIV-Positive Mothers and Their Children

Mother		1st child		2nd child		3rd child	
No.	Genotype[a]	HIV[b]	Genotype	HIV	Genotype	HIV	Genotype
1	w.t./ Δ	neg.	Δ / Δ				
2	w.t./ Δ	neg.	w.t./w.t.				
3	w.t./w.t.	neg.	w.t./ Δ	neg.	w.t./w.t.	neg.	w.t./w.t.
4	w.t./w.t.	neg.	w.t./w.t.	neg.	w.t./w.t.		
5	w.t./w.t.	neg.	w.t./w.t.	**pos.**	w.t./w.t.		
6	w.t./w.t.	**pos.**	w.t./w.t.	neg.	w.t./ Δ		
7	w.t./w.t.	**pos.**	w.t./w.t.				
8	w.t./w.t.	**pos.**	w.t./w.t.				
9	w.t./w.t.	**pos.**	w.t./w.t.				
10	w.t./w.t.	neg.	w.t./ Δ				
11	w.t./w.t.	neg.	w.t./w.t.				
12	w.t./w.t.	neg.	w.t./w.t.				

[a] *w.t.,* wild-type CCR5 allele; Δ, mutant Δ32ccr5 allele.
[b] *neg.,* uninfected; *pos.,* infected by vertical transmission.

a genotypic distribution consistent with the prediction of the Hardy-Weinberg equation.

The results provide additional evidence that Δ32ccr5 homozygosity may contribute to protection against infection with HIV-1 since none of the HIV-infected individuals (adults or children) exhibit this genotype (the *P* value calculated for the comparison of Δ32ccr5 homozygotes among infected and uninfected individuals in this study was 0.055).

Evidence on whether heterozygosity may also confer some degree of protection is not clear. A partially protective effect of a heterozygous genotype was suggested by a European study [Samson et al., 1996] but not by two investigations on populations from the United States [Dean et al., 1996; Huang et al., 1996]. The issue may be complicated by varying degrees of protection for different routes of transmission and an increase of heterozygotes within a cohort over time due to a slower disease progression of these individuals. In addition, the genetic influx of non-Caucasian origin may vary among groups and is difficult to estimate. Investigating a single defined transmission mode within a Caucasian population, as described above, may therefore be helpful to clarify this question.

The data indeed suggest an influence of Δ32ccr5 on vertical transmission since the mutant allele frequency in the group of children infected vertically is significantly lower than that for the other groups investigated. A correlation of the CCR5/Δ32ccr5 heterozygous genotype with the rate of vertical transmission could be caused either at the level of the infants (higher resistance of the foetus to infection) or at the maternal level (heterozygous mothers may be less infectious, e.g., due to lower viral load) or a combination of both. Future studies on larger numbers of mother–child pairs are necessary. In any case, it is tempting to suggest that an influence of the Δ32ccr5 allele may, among other factors, contribute to the lower vertical transmission rate observed in white vs. black populations [European Collaborative Study 1995; Mayaux et al., 1997].

The influence of the Δ32ccr5 allele on vertical transmission rates should be evaluated in the context of other well-established risk factors, such as viral load and disease progression of the mother, breast-feeding, and social and nutritional status. If future evidence confirms the above observations, genetic screening of parents and children may yield useful information for prognosis and the planning of therapeutic or obstetric intervention.

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